Soil Organic Carbon and Nitrogen Accumulation in Plots of Rhizoma Perennial Peanut and Bahiagrass Grown in Elevated Carbon Dioxide and Temperature

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ABSTRACT

Carbon sequestration in soils might mitigate the increase of carbon dioxide (CO_2) in the atmosphere. Two contrasting subtropical perennial forage species, bahiagrass (BG; *Paspalum notatum* Flügge; C_4), and rhizoma perennial peanut (PP; *Arachis glabrata* Benth.; C_3 legume), were grown at Gainesville, Florida, in field soil plots in four temperature zones of four temperature-gradient greenhouses, two each at CO_2 concentrations of 360 and 700 μmol mol^{-1}. The site had been cultivated with annual crops for more than 20 yr. Herbage was harvested three to four times each year. Soil samples from the top 20 cm were collected in February 1995, before plant establishment, and in December 2000 at the end of the project. Overall mean soil organic carbon (SOC) gains across 6 yr were 1.396 and 0.746 g kg^{-1} in BG and PP, respectively, indicating that BG plots accumulated more SOC than PP. Mean SOC gains in BG plots at 700 and 360 μmol mol^{-1} CO_2 were 1.450 and 1.343 g kg^{-1}, respectively (not statistically different). Mean SOC gains in PP plots at 700 and 360 μmol mol^{-1} CO_2 were 0.949 and 0.544 g kg^{-1}, respectively, an increase caused by elevated CO_2. Relative SON accumulations were similar to SOC increases. Overall mean annual SOC accumulation, pooled for forages and CO_2 treatments, was 540 kg ha^{-1} yr^{-1}. Eliminating elevated CO_2 effects, overall mean SOC accumulation was 475 kg ha^{-1} yr^{-1}. Conversion from cropland to forages was a greater factor in SOC accumulation than the CO_2 fertilization effect.

S EQUESTRATION of carbon in the soil is one method of potentially decreasing the amount of carbon dioxide (CO_2) in the atmosphere and mitigating the potential impact of global warming by rising levels of greenhouse gases (Rosenzweig and Hillel, 2000; Izaurralde et al., 2001; Metting et al., 2001; Lal, 2004). During the 1990s, several reviews evaluated the relationships of soils and global change (Lal et al., 1995) and the role of soil processes in the global carbon cycle (Lal et al., 1998a). Similar reviews evaluated the potential of management of agricultural lands for carbon sequestration in soils (Lal et al., 1998b). For the most part, these reviews and analyses focused more on cropland management and forests than on grassland systems in assessing the potential for soil carbon sequestration. However, globally averaged, grassland ecological zones contain more soil carbon (averaging of approximately 24 kg C m^{-2} or totaling approximately 480 Pg C in long-term resistant soil carbon plus humus) than temperate or tropical forests (Greenland, 1995).

Measurements of the rates of accumulation of soil organic matter (SOM) have been made. Potter et al. (1999) reported that carbon accumulated at the rate of 447 kg C ha^{-1} yr^{-1} over a 60-yr period when degraded land in Texas was returned to grassland. Garten and Wullschleger (2000) predicted a 12% increase in the soil carbon inventory over a 10-yr period following establishment of switchgrass (*Panicum virgatum* L.). Post and Kwon (2000) calculated that average accumulation rates of soil organic carbon (SOC) after establishment of a forest or pasture would be 338 and 332 kg C ha^{-1} yr^{-1}, respectively, virtually identical rates.

Models have been developed to predict the effect of management practices on soil carbon sequestration. Qian et al. (2003) used the CENTURY ecosystem model for predicting the impact of clipping removal and nitrogen fertilization on home lawn conditions of Kentucky bluegrass (*Poa pratensis* L.) in Colorado. They found that returning clippings to the soil surface would increase soil carbon sequestration under both high- and low-nitrogen fertilization.

Not all studies indicate that landscapes are accumulating soil carbon. Bellamy et al. (2005) reported that, across all soils in England and Wales, soil carbon decreased at the rate of 0.6% per year over the period 1978–2003, and suggested that losses in temperate regions are likely to be offsetting absorption of carbon by terrestrial sinks. More research and synthesis of information is needed to resolve these uncertainties regarding soil carbon accumulation.

While considerable work has been done on productivity and sequestration of carbon by natural grassland ecosystems (e.g., Post and Kwon, 2000; Schuman et al., 2002; Van Groenigen et al., 2002; Brye and Kucharik, 2003; Morgan et al., 2004; Pendall et al., 2004), less research has been conducted on managed systems, especially in the Southeastern United States (Franzluebbers et al., 2000; Franzluebbers and Stuedemann, 2003). However, perennial forage crops and pastures in humid

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Abbreviations: BG, bahiagrass; PP, rhizoma perennial peanut; SOC, soil organic carbon; SOM, soil organic matter; SON, soil organic nitrogen; TGG, temperature-gradient greenhouse.

areas could contribute significantly to carbon sequestration. In a review, Franzluebbers (2005) reported mean values of 1030 ± 900 kg C ha^{-1} yr^{-1} obtained from 10 citations. However, some of the citations included carbon accumulation data where broiler litter was applied. The mean of values without broiler litter application with a study duration of 10 yr or longer gave a mean value of 522 ± 276 kg C ha^{-1} yr^{-1}, a more likely mean organic carbon sequestration rate. Alternatively, a derived median of all the reported values without broiler litter was 565 kg C ha^{-1} yr^{-1}. Soil organic nitrogen (SON) changes should accompany any changes in SOC because the two elements are linked in both plant inputs and in eventual humic and fulvic acid substances in long-term SOM (Wander, 2004).
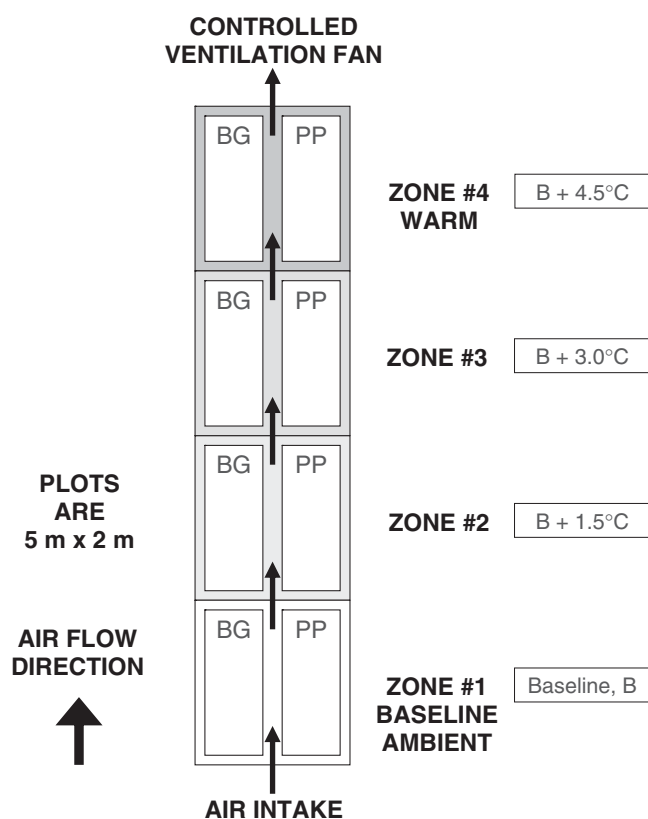
A few studies have investigated the impact of elevated CO_2 or elevated temperature, or the combination of each factor, on growth responses and the accumulation of soil carbon by grassland systems. Some of the studies also included nitrogen fertilization and water treatments as well. In a 4-yr microcosm study of the C_3 grass *Danthonia richardsonii* cv. Cashmore at Canberra, Australia, Lutze and Gifford (1998) found soil carbon gain in the soil pool was increased with CO_2 enrichment (718 μmol mol^{-1} versus 359 μmol mol^{-1}) by ratios of 1.15, 1.32, and 1.57 for low, mid, and high N treatment levels (22, 67, and 198 kg N ha^{-1} yr^{-1}, respectively). Based on an initial soil carbon content of 350 g C m^{-2}, this translates to about 130, 280, and 500 kg C ha^{-1} yr^{-1} enhancement of soil carbon at elevated CO_2 over the 4-yr period. These increases in soil carbon accumulation were accompanied by increases of shoot and root biomass accumulations. Increases of mean global surface temperatures for the last half-century have been about 0.1°C per decade for the daily maximum, but about 0.2°C per decade (doubled) for the daily minimum (Easterling et al., 1997; Folland et al., 2001). Using temperature-gradient tunnels, Volder et al. (2004) investigated the effect of ambient and 750 μmol mol^{-1} CO_2 in combination with ambient temperature, a constant warming of 3.0°C, and a daytime warming of 2.2°C with a nighttime warming of 4.0°C on the growth of bulbous Canary grass (*Phalaris aquatica* L. cv. Holdfast) swards. Over a 20-mo period in two growing seasons, they found that elevated CO_2 increased total aboveground biomass by 11%, but there was no effect of either warming treatment, and no evidence that high nighttime temperature had any different effect than constant warming. Li et al. (2004) modeled soil carbon gains for semiarid, ungrazed grassland near Lethbridge, Alberta, Canada to 2100 using current climate conditions and obtained a linear annual rate of soil carbon gain of 260 kg C ha^{-1} yr^{-1}. However, their model indicated only about 20 kg C ha^{-1} yr^{-1} more soil carbon gain for predicted climate change conditions (elevated CO_2, temperature, and rain). Xie et al. (2005) found CO_2 enrichment increased new carbon inputs to the soil of 1030, 1340, and 755 kg C ha^{-1} yr^{-1} for three conditions over a 9-yr period in the Swiss FACE grassland experiment. However, the higher input of "new" carbon apparently caused an increased decomposition of "old" carbon, which led them to conclude that the

potential of carbon sequestration in established, well managed grassland would be low. In a grassland field experiment in Minnesota, USA, Dijkstra et al. (2005) found that increased biomass accumulation with elevated CO_2 mostly affected SOM pools with fast turnover rates (labile C, microbial biomass), but had no significant effect on total soil C and N pools, or the decomposition of the more recalcitrant C. During a 5-yr study using open-top chambers, Pendall et al. (2004) found that rhizodeposition (addition of carbon from roots to soil carbon pools) in the shortgrass steppe region of northeastern Colorado, USA, was 830 ± 160 and 350 ± 90 kg C ha^{-1} yr^{-1} in elevated (720 μmol mol^{-1}) versus ambient (360 μmol mol^{-1}) treatments, respectively. This study in semiarid grassland seemed to provide the most unambiguous indication of the potential for elevated CO_2 to stimulate the accumulation of soil carbon. Furthermore, Milchunas et al. (2005) reported that these elevated CO_2 treatments caused 52% greater root-length growth, 37% greater root-length losses, and 41% greater total pool sizes. Finally, Parr and Sullivan (2005) investigated the role of organic carbon occluded within phytoliths ("PhytOC") in carbon sequestration. They found the percentage of SOM as PhytOC ranged from about 1% near the surface to as high as 82% deeper in profiles of paleosols sampled in Papua New Guinea. From one set of calculations, they reported that a sugarcane (*Saccharum officinarum* L.) crop produced 181 kg C ha^{-1} yr^{-1} of PhytOC, which is in the low end of the range of soil carbon accumulation rate reported for many types of grasslands. This PhytOC might be a significant sink of carbon for grass species that are not typically useful in grazing lands because of high phytolith content in leaves.

We hypothesized that growth of perennial forage crops in humid subtropical environments on previously cultivated land will increase accumulation of SOC and SON and, furthermore, that accumulation of SOC and SON will be enhanced by elevated CO_2 and diminished by increasing temperature, with specific responses dependent on characteristics of the forage crop species. To address these hypotheses, the first objective was to determine the amounts of SOC and SON that would be stored across time after establishment of two forage species, bahiagrass (BG; a stoloniferous C_4 photosynthetic-pathway grass), and rhizoma perennial peanut (PP; a rhizomatous C_3 photosynthetic-pathway legume) in a Florida sandy soil previously under annual crop (small grains and maize) cultivation for more than 20 yr. The second objective was to determine the impact of global climate change factors (rising CO_2 concentration and elevated temperatures) on accumulation of SOC and SON by these two different forage species in this humid, subtropical environment. Soil organic nitrogen was included in this study because of the association of nitrogen in the SOC pools ranging from fresh plant materials to stable humic and fulvic acid fractions of stable SOM. Furthermore, studies indicate that nitrogen inputs play an important role in soil carbon accumulation (Lutze and Gifford, 1998; Van Groenigen et al., 2002; Brye and Kucharik, 2003; Qian et al., 2003).

MATERIALS AND METHODS

On 9 Apr. 1995, 'Pensacola' bahiagrass seeds were sown and 'Florigraze' rhizoma perennial peanut rhizomes were incorporated into the soil (propagated vegetatively) in four computer-controlled temperature-gradient greenhouses (TGG) as described by Boote et al. (1999), Fritschi et al. (1999b), and Newman et al. (2001). The supervisory control and data acquisition (SCADA) system consisted of FIX DMACS software (Intellution, Norwood, MA) that controlled Keithley Metrabyte hardware (Keithley Instruments, Cleveland, OH) consisting of temperature input boards for type-t (copper-constantan) thermojunctions, analog input–output boards, and digital on–off switches. The FIX DMACS control program was executed by use of a MS-DOS based PC connected to the Keithley Metrabyte hardware system by an optically isolated computer input–output card. Each TGG, 27.45 m long and 4.27 m wide, was placed over natural field soil profile (Millhopper fine sand, a loamy, siliceous, hyperthermic Grossarenic Paleudult). Each TGG consisted of an entry zone (3.66 m long), four experimental zones (each 5.49 m long), and one exit zone (1.83 m long) along the length (Fig. 1). Each of the four experimental zones were maintained at step increases of temperature (baseline temperature, +1.5, +3.0, and +4.5°C, respectively) by use of computer-controlled injections of heated air (upwind

CONTROLLED VENTILATION FAN

ZONE #4 WARM — B + 4.5°C

ZONE #3 — B + 3.0°C

ZONE #2 — B + 1.5°C

ZONE #1 BASELINE AMBIENT — Baseline, B

BG   PP

PLOTS ARE 5 m x 2 m

AIR FLOW DIRECTION

AIR INTAKE

Fig. 1. Temperature-gradient greenhouse (TGG) layout illustrating 5- × 2-m field-soil plots of bahiagrass (BG) and rhizoma perennial peanut (PP) in four temperature zones. Each TGG consisted of an entry zone (3.66 m long, not shown), four experimental zones (each 5.49 m long), and one exit zone (1.83 m long, not shown). Arrows indicate the direction of air flow. Heated air was introduced between Zones 1 and 2, 2 and 3, and 3 and 4 to provide step-change in temperature, in conjunction with a variable speed exhaust fan. Both the ventilation fan and the heated air inputs were under computer control. A CO$_2$ injection system (not illustrated) was provided for two of the four TGGs.

of Zones 2, 3, and 4) and a controlled variable-speed ventilation fan. The temperature difference of 4.5°C from the first zone to the fourth zone was maintained by the SCADA system by detecting temperatures and energizing actuators controlling the ventilation fan and the heaters. Solar radiation substituted for part of the heated air injections during bright sunlight conditions. Daytime CO$_2$ treatments were 360 (ambient) and 700 μmol mol^{-1}, on a mole fraction basis [i.e., μmol (CO$_2$) mol^{-1} (atmospheric air)], and maintained by controlled injection of CO$_2$ for 6 yr from 1995 through 2000. Examples of the control level of temperature and CO$_2$ were given by Boote et al. (1999) and Newman et al. (2001, 2006).

Paired plots of each species in each zone were 5 by 2 m. The design was a split strip-strip plot; CO$_2$ was the whole plot factor and was allocated to greenhouses at random with two greenhouses per level of CO$_2$ (two replications of CO$_2$ treatments). Fertilizer was applied as small frequent split applications according to plan during each year of growth based on soil analyses and needs for herbage production. In 1995, a total of 80, 42, and 149 kg ha^{-1} of N, P, and K were applied to both species (Fritschi et al., 1999b; Boote et al., 1999). Fertilizer applied to both species totaled 70, 30, and 58 kg ha^{-1} of N, P, and K in 1996; 80, 36, 173, 65, and 128 kg ha^{-1} of N, P, K, Mg, and S in 1997; and 80, 36, 152, 52, and 103 kg ha^{-1} of N, P, K, Mg, and S in 1998 and 1999 (Boote et al., 1999; Newman et al., 2001). Also in 1998 and 1999 BG plots were split in two parts with one-half of each plot receiving a total of 320 kg ha^{-1} of N (Newman et al., 2006). In 2000 the amount of fertilizer applied was 60, 27, 125, 37, and 75 kg ha^{-1} of N, P, K, Mg, and S, with the split BG plots receiving an additional 155 kg ha^{-1} of N (215 kg ha^{-1} of N). Irrigation was applied two to three times weekly at rates designed to exceed evapotranspiration rates slightly in the warm zones of each greenhouse. The amount of irrigation was 7 to 8 mm d^{-1} on average during the 8- to 9-mo period of rapid growth, and about half that rate during winter months. The total average annual irrigation was 2020 mm yr^{-1} (Boote et al., 1999), which is about 60% above Gainesville average annual rainfall. Plant herbage was mowed and removed three to four times during each growing season from 1995 through 2000. Details of the treatment conditions, fertilizer applications, irrigation, plant responses, and herbage yield and quality data were reported earlier (Boote et al., 1999; Fritschi et al., 1999a, 1999b; Newman et al., 2001, 2005, 2006). Average daily mean ambient temperatures measured with a solar-radiation-shielded, aspirated thermocouple ranged from 11°C for the month of January 1996 (Boote et al., 1999; Newman et al., 2001) to 28°C for the month of June 1998 (Newman et al., 2006). All environmental data (including air temperatures in the four zones of each TGG, ambient air temperature, CO$_2$ concentrations, and photosynthetically active radiation) and control system data were recorded at 1-min intervals. The ambient temperatures were consistently about 1.5°C lower than the temperatures of the first zone of each TGG, so the actual temperatures experienced by the plants inside were 1.5, 3.0, 4.5, and 6.0°C above Gainesville ambient temperatures for the first (baseline), second, third, and fourth zones, respectively (Boote et al., 1999; Newman et al., 2001, 2005, 2006).

Four replicated soil samples from the top 20 cm of each plot were collected in February 1995 before forage establishment, and again every February before new growth began (1996, 1997, 1998, 1999, 2000), and in December 2000 when the study ended. For each of the four replicated soil samples, there were nine systematic subsamples over the entire 2- × 5-m plot area. The soil was air-dried in a laboratory and then was sieved to 2.2 mm to remove residues of plant particulate organic matter. We are reporting the SOC and SON from February 1995 and December 2000, to bracket effects of 6 yr of treatments.

Soil samples were shipped to Pendleton, OR, for analyses. Samples were then re-dried at 40°C for 24 h and sieved in a round sieve having 2.2-mm circular holes (Seedburo, Chicago, IL). Subsamples were roller-milled for 4 h per subsample (Smith and Um, 1990). This soil was analyzed for total C and N in a Thermo Finnigan FlashEA 1112 CNS analyzer (CE Elantech, Lakewood, NJ) fitted with an autosampler. The sample was oxidized at 1800°C. The gases (CO_2 and NO_x) were passed through a reducing column (Cu), dried over anhydrous magnesium perchlorate, separated in a 2-m (5-mm i.d.) steel GC column packed with HayeSep polymer, and then measured with a thermoconductivity detector. Results of the C and N analysis of the roller-milled soil, based on three laboratory replicates of each sample, proved variable, so approximately half of each roller-milled subsample was ball-milled in a Model 8000 mill (SPEX, Metuchen, NJ), for 2 min per subsample. This yielded acceptable laboratory variability in C and N. No inorganic soil carbon was detected in a random selection of samples. Also, no nitrite was detected, but nitrate was detected at low levels of 0.0002 to 0.0015 g (N) kg^{-1} (soil), and ammonia N was detected at 0.0040 to 0.0137 g (N) kg^{-1} (soil). The highest levels detected represented about 5% of the mean soil nitrogen in the February 1995 samples and about 3.7% of the mean soil nitrogen in the December 2000 samples. Therefore, no correction was undertaken for inorganic carbon or nonorganic nitrogen in the data set.

Statistical analyses were conducted for the complete set ($n = 128$ for C and for N) of February 1995 and December 2000 data by SAS PROC GLM (SAS Institute, 1996). Since the objective was to determine the treatment effects across the 6 yr, statistical analyses were repeated for the differences in the values (accumulations or gains) between the last and first samplings ($n = 64$ for overall species effects and CO_2 effects, and $n = 32$ for species × CO_2 interaction effects and for temperature effects).

RESULTS AND DISCUSSION

Overall Effect of Forage on Soil Organic Carbon and Soil Organic Nitrogen

Across 6 yr in all treatments, SOC increased from 4.185 to 5.266 g kg^{-1} (1.081 g kg^{-1}), an increase of 26% (Fig. 2A). The 6 yr of treatment (time effect) caused differences that were significant for both SOC and SON ($P < 0.01$) (Table 1). The change in SOC was equivalent to an SOC increase of 0.180 g kg^{-1} yr^{-1} or 4.3% per year. Similarly, SON increased from 0.277 to 0.372 g kg^{-1} (0.095 g kg^{-1}), an increase of 34% (Fig. 2B), and an increase of 0.016 g kg^{-1} yr^{-1} or 5.7% per year. Both SOC and SON increased, so we conclude that forage crops have the potential to enhance soil carbon accumulation. The overall SOC to SON ratio was 15.1 at the beginning of the experiment and 14.2 at the end of the experiment. Percentage increases of SON were greater than SOC, which, coupled with the decreased C to N ratio, indicated that a thorough, continuous breakdown of newly deposited rhizosphere SOM must have been occurring across the 6-yr period. Apparently, the recently incorporated C compounds were easily oxidized to CO_2 by microbial activity, while the N was readily integrated in an organic fraction that was reticent to decomposition or mineralization. The first hypothesis was supported; that is, growth of perennial forage crops in humid subtropical environments on previously cultivated land increases the accumulation of SOC and SON.

Species Effect for Bahiagrass and Rhizoma Perennial Peanut on Increase of Soil Organic Carbon and Soil Organic Nitrogen across Six Years

Soil organic carbon increased by 1.396 g kg^{-1} for BG and 0.746 g kg^{-1} for PP, a BG to PP ratio of 1.87 (Fig. 2C). Likewise, SON increased by 0.1118 g kg^{-1} for BG and 0.0765 g kg^{-1} for PP, a BG to PP ratio of 1.46 (Fig. 2D). The effect of species was significantly different ($P < 0.01$) for both SOC and SON. The SOC to SON ratios for the increases were 12.5 for BG and 9.75 for PP. Thus, the C to N ratios of accumulated SOM under both species were lower than the original C to N ratio of 15.1. The lower C to N ratio of PP likely results from lower C to N ratio of the legume belowground tissues than the grass tissues. Bahiagrass sod residue to 20-cm depths has a C to N ratio of 55 based on calculations from N content data of Jackson (2003). Root C to N ratios were somewhat higher (60 to 70) as reported by Pendall et al. (2004) for a semiarid grassland and by Jastrow et al. (2000) for a tallgrass prairie, but tallgrass prairie rhizome C to N ratios were somewhat lower (40 to 50) according to Jastrow et al. (2000). We conclude that BG promotes the accumulation of SOC more than does PP, even though herbage yields were higher for PP than for BG (previously reported by Fritschi et al., 1999a, 1999b; Newman et al., 2001, 2005). However, measured belowground biomass (total root–stolon–rhizome) in the third year (1997) of BG and PP (pooled across CO_2 and temperature treatments) was 2315 and 1304 g m^{-2}, respectively (Boote et al., 1999). This belowground biomass ratio of 1.78 is consistent with the BG to PP accumulated SOC ratio of 1.87 after 6 yr. The BG has a higher C to N ratio than the legume PP (Fritschi et al., 1999a), so the decomposition of BG belowground biomass would be expected to be slower than decomposition of PP belowground biomass. This finding supports part of the second hypothesis that specific SOC and SON accumulation responses depend on characteristics of the forage crop species.

Carbon Dioxide Effect on Increase of Soil Organic Carbon and Soil Organic Nitrogen at 360 and 700 μmol mol^{-1} across Six Years

Soil organic carbon increased by 0.943 g kg^{-1} for 360 μmol mol^{-1} and 1.199 g kg^{-1} for 700 μmol mol^{-1} CO_2 across all treatments, a ratio of 1.27 (Fig. 2E). Likewise, SON increased by 0.0886 g kg^{-1} for 360 μmol mol^{-1} and 0.0997 g kg^{-1} for 700 μmol mol^{-1}, a ratio of 1.13 (Fig. 2F). The effect of elevated CO_2 was significant for SOC ($P < 0.01$). The SOC to SON ratios for the increases were 10.9 for the 360 μmol mol^{-1} CO_2 treatment plots and 12.3 for the 700 μmol mol^{-1} CO_2 treatment plots. Thus, the ratio of the C to N ratios of the SOM increases of elevated versus ambient CO_2 treatments is 1.13. Increase in this ratio is generally comparable with the increase of C to N ratio of about 0.99, 1.13, 1.15, and 1.26 for root biomass reported by Pendall
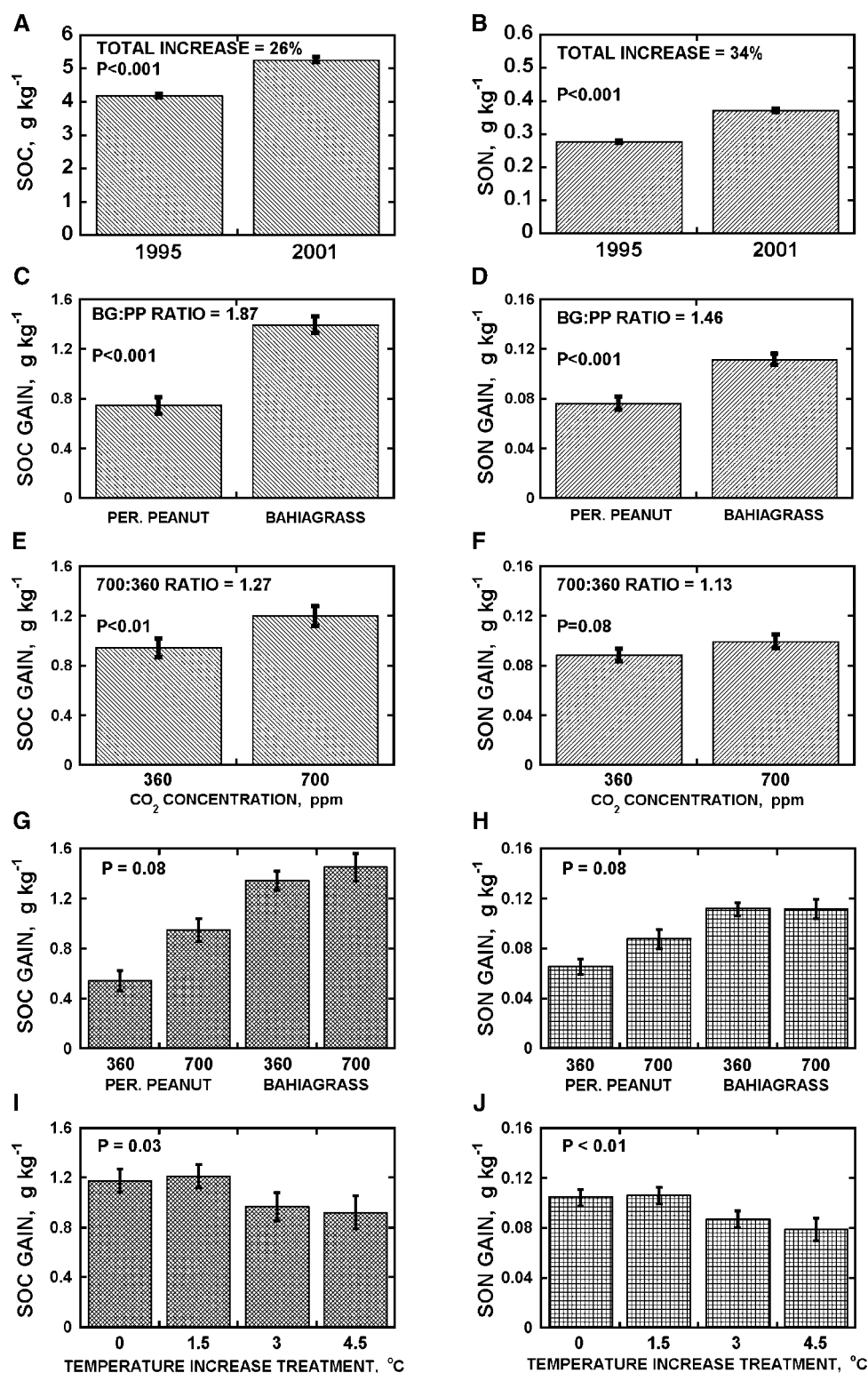
Fig. 2. Comparisons of changes in soil organic carbon (SOC) and soil organic nitrogen (SON) before and after the 6-yr experimental period (1995 through 2000) at Gainesville, Florida. Total (A) soil organic carbon (SOC) and (B) soil organic nitrogen (SON) concentrations at the beginning and at the end of the experiment pooled for all CO_2, temperature, and species treatments (the data plotted as 2001 were actually sampled in December 2000). Mean 6-yr gains in (C) SOC and (D) SON in plots of rhizoma perennial peanut (PP) and bahiagrass (BG) pooled for CO_2 and temperature treatments. Comparison of the overall effect of two levels of carbon dioxide concentration (360 and 700 μmol mol^{-1}, indicated by ppm, mole fraction) on gains of (E) SOC and (F) SON pooled for temperature and species treatments. Comparison of the $CO_2 \times$ species interaction effects on (G) SOC and (H) SON gains pooled over temperature treatments. Comparison of the overall effect of four levels of temperature treatments on gains of (I) SOC and (J) SON pooled for CO_2 and species treatments. Error bars indicate standard deviations of the means.

Table 1. Statistical *P* values from analysis of variance of soil organic carbon and soil organic nitrogen concentration using data for both beginning and end of experiment (both years) and using difference in concentration data (difference = ending concentration minus beginning concentration).

Source of variation	Soil organic carbon		Soil organic nitrogen	
	Both years	Difference	Both years	Difference
Year	<0.01	–	<0.01	–
Species	<0.01	<0.01	<0.01	<0.01
Year × species	<0.01	–	<0.01	–
CO$_2$	<0.01	<0.01	<0.01	0.08
Year × CO$_2$	<0.01	–	0.11	–
Species × CO$_2$	0.01	0.08	<0.01	0.08
Year × species × CO$_2$	0.09	–	0.11	–
Temperature	<0.01	0.03	<0.01	<0.01
Year × temperature	0.04	–	0.01	–
Species × temperature	0.97	0.12	0.95	0.17
CO$_2$ × temperature	0.02	0.13	0.28	0.30
Year × species × temperature	0.15	–	0.19	–
Year × CO$_2$ × temperature	0.16	–	0.34	–
Species × CO$_2$ × temperature	0.58	0.69	0.68	0.69
Year × species × CO$_2$ × temperature	0.72	–	0.70	–

et al. (2004) for the second, third, fourth, and fifth year of a 5-yr experiment of CO$_2$ enrichment in a semiarid grassland, which reflects the frequent observations that elevated CO$_2$ promotes higher C to N ratios of plant tissues. We conclude that elevated CO$_2$ (700 μmol mol^{-1}) caused greater accumulation of SOC than ambient CO$_2$ (360 μmol mol^{-1}), as was expected. Previous reports showed that elevated CO$_2$ produced more herbage yield for both the C$_3$ species PP and the C$_4$ species BG (Fritschi et al., 1999a, 1999b; Newman et al., 2001, 2005). The impact of elevated CO$_2$ on SON was much smaller (*P* = 0.08). These findings support one component of the second hypothesis, that accumulation of SOC and SON will be enhanced by elevated CO$_2$.

In addition to the main effects, species × CO$_2$ interaction effects on the increase of SOC and SON are particularly relevant in this study. Soil organic C increased by 0.544 g kg^{-1} for PP at 360 μmol mol^{-1}, by 0.949 g kg^{-1} for PP at 700 μmol mol^{-1} CO$_2$, by 1.343 g kg^{-1} for BG at 360 μmol mol^{-1}, and by 1.450 g kg^{-1} for BG at 700 μmol mol^{-1} CO$_2$ (Fig. 2G). Thus, the SOC ratio of PP700/PP360 = 1.74, and the SOC ratio of BG700/BG360 = 1.08. Likewise, SON increased by 0.0655 g kg^{-1} for PP at 360 μmol mol^{-1}, by 0.0875 g kg^{-1} for PP at 700 μmol mol^{-1} CO$_2$, by 0.1117 g kg^{-1} for BG at 360 μmol mol^{-1}, and by 0.1118 g kg^{-1} for BG at 700 μmol mol^{-1} CO$_2$ (Fig. 2H). Thus, the SON ratio of PP700/PP360 = 1.34, and the SON ratio of BG700/BG360 = 1.00. The SOC to SON ratios were 8.31, 10.8, 12.0, and 13.0 for PP at 360 μmol mol^{-1}, PP at 700 μmol mol^{-1}, BG at 360 μmol mol^{-1}, and BG at 700 μmol mol^{-1}, respectively. Thus, the ratio of the C to N ratios was considerably larger for the accumulated SOM of the PP under CO$_2$ enrichment versus ambient CO$_2$ (1.30) than it was for BG (1.08). We conclude that elevated CO$_2$ caused a relatively large increase of SOC accumulation in PP but a relatively small increase of SOC accumulation in BG. Elevated CO$_2$ caused an increase of

SON accumulation in PP but had no effect on SON accumulation in BG. The evidence for a significant species × CO$_2$ interaction is weak for both SOC and SON (*P* = 0.08) (Table 1).

The large CO$_2$ effect on SOC and SON of PP and the small or nonexistent CO$_2$ effect on SOC and SON of BG are supported by differential CO$_2$ enhancement of aboveground and belowground dry matter for these species (Fig. 3). For the combination of 1996 and 1997 data, the belowground root–rhizome of PP was increased 21.6% by 700 μmol mol^{-1} elevated CO$_2$ while that for BG was only 6.3% greater (Fig. 3 and Boote et al., 1999); these belowground responses are consistent with the C$_3$ versus C$_4$ nature of the species and with the degree of CO$_2$ enhancement of herbage yield of the two species. Regardless of the CO$_2$ effect, the greater increase in SOC and SON for BG was dominated by the 78% greater belowground biomass of BG by the third year (1997) (BG averaged 2315 g m^{-2} versus 1304 g m^{-2} for PP; Fig. 3 and Boote et al., 1999).

Effect of a Range of Four Temperatures on Accumulation of Soil Organic Carbon and Soil Organic Nitrogen

During the 6 yr, and across all treatments, SOC increased by 1.177, 1.214, 0.970, and 0.924 g kg^{-1} when forages were grown at baseline (0), +1.5, +3.0, and +4.5°C temperature treatments, respectively (Fig. 2I). Likewise, SON increased by 0.1044, 0.1060, 0.0871, and 0.0790 g kg^{-1} for baseline (0), +1.5, +3.0, and +4.5°C temperature treatments, respectively (Fig. 2J). The effect of temperature was significant on both SOC (*P* = 0.03) and on SON (*P* < 0.01). Thus, both SOC and SON increased more at the cooler temperatures of the temperature gradient than at the warmest temperatures of the temperature gradient. The SOC to SON ratios were 11.3, 11.5, 11.1, and 11.7 for the baseline (0), +1.5, +3.0 and +4.5°C temperature treatments, respectively, indicating that temperature had little effect on C to N
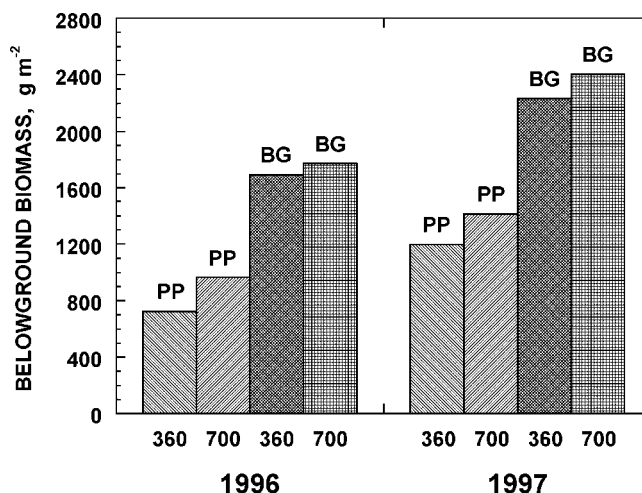
Fig. 3. Belowground biomass in 1996 and 1997 of perennial peanut (PP) and bahiagrass (BG) grown under 360 or 700 μmol mol^{-1} (indicated by ppm, mole fraction) CO$_2$ for comparison with Fig. 2C and 2D and Fig. 2G and 2H. Adapted from Boote et al. (1999).

ratios of the accumulated SOM. Probably this relatively small air temperature range was not great enough to cause a temperature effect on C to N ratios of accumulated SOM (soil temperature gradients were not as large as air temperature gradients, data not available). Kandeler et al. (1998) found that root C to N ratios of four species were not affected by a 2°C increase in temperature at ambient CO_2, with more complex responses for elevated CO_2. We conclude that slight increases above Gainesville, Florida, ambient temperatures would not change SOC and SON accumulation by these forages. However, large increases in temperature would diminish SOC and SON accumulation. This trend occurred despite earlier reports that herbage yields increased continuously across all temperature treatments (Boote et al., 1999; Fritschi et al., 1999b; Newman et al., 2001). These findings support the hypothesis that accumulation of SOC and SON will be diminished by increasing temperature, especially for 3°C or more above Gainesville ambient temperatures.

Comparisons with Other Soil Organic Carbon Reports

From our data we calculated a SOC accumulation of 540 kg C ha^{-1} yr^{-1} from the 6 yr of data. Removing the CO_2 effect, by dividing by 1.27, yielded 425 kg C ha^{-1} yr^{-1} of SOC. As expected, SON accumulation paralleled SOC accumulation. Albrecht (1938) reported an accumulation of 380 kg C ha^{-1} yr^{-1} in a 14-yr red clover study in Missouri. Lutze and Gifford (1998) found about 130, 280, and 500 kg C ha^{-1} yr^{-1} enhancement of SOC accumulation at elevated CO_2 at low, medium, and high nitrogen fertilization over a 4-yr period, accompanied by increases of root biomass accumulations. Potter et al. (1999) showed an accumulation of 450 kg C ha^{-1} yr^{-1} for a 60-yr study of degraded Texas prairie soils returned to grasslands. Pendall et al. (2004) found rhizodeposition in the shortgrass steppe region of northeastern Colorado was 830 ± 160 and 350 ± 90 kg C ha^{-1} yr^{-1} in elevated (720 μmol mol^{-1}) versus ambient (360 μmol mol^{-1}) treatments, respectively. We calculated a value of 522 ± 276 kg C ha^{-1} yr^{-1} from data in a review paper by Franzluebbers (2005). Allen and Nelson (2006) reported 370 kg ha^{-1} yr^{-1} accumulation of SOC across 15 yr for perennial peanut, which is consistent with perennial peanut accumulating less SOC than bahiagrass. Finally, our data showing SOC and SON accumulation rates are clearly comparable with many studies reported in the introduction.

CONCLUSIONS

Conversion from cropland to perennial forages was a greater factor in SOC accumulation than the CO_2 fertilization effect itself. The BG plots gained more SOC than PP plots, which is in agreement with the fact that more belowground biomass was found in the BG plots. On the other hand, SOC accumulation in PP plots, but not BG plots, was responsive to elevated CO_2, which would be expected since C_3 plant photosynthesis and biomass production is more responsive to elevated CO_2 than C_4 plant photosynthesis. Our data showing SOC and SON annual accumulation rates are clearly comparable with many studies reported in the introduction. Subtropical perennial forage species growing in humid climates appear to be capable of accumulating SOC at rates comparable with other grasslands in other climates, and therefore they should have the potential for sequestering meaningful amounts of SOC in comparison with other agroecosystems.

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